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DEOXYRIBONUCLEIC ACID

Translation No. 1482

JULY 1965

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## SCATTERING OF LIGHT BY SOLUTIONS OF NATIVE, DENATURED AND DEGRADED DEOXYRIBONUCLEIC ACID

[Following is the translation of an article by V. Ye. Eskin and O. Z. Korotkina, Institute of High Molecular Compounds, USSR Academy of Sciences, Leningrad, published in the Russian-language periodical Biofizika (Biophysics), Vol X, No 1, 1965, pages 26--31. It was submitted on 22 July 1963. Translation performed by Sp/7 Charles T. Ostertag Jr.]

Light-scattering is known as one of the main physical methods of investigating the structure of macromolecules. Measurements of the angular intensity distribution of scattered light ( $\bar{I}_\theta$ ), interpreted according to the method of double extrapolation  $[\bar{I}]$  (to the zero angle of scattering  $\theta$  and the zero concentration  $c$ ), produce the same unique possibility; without any preliminary assumptions concerning the structure of the particle, they make it possible to determine, according to the initial slope of the plot of dependency  $(c/\bar{I}_0)_{c \rightarrow 0}$  on  $\sin^2 \theta/2$ , the root mean square radius of gyration  $(\bar{R}^2)^{1/2}$ , which is an important characteristic of its structure.

However, the application of such a method for the study of molecules of native deoxyribonucleic acid (DNA) encounters several difficulties, connected with the large dimensions of these macromolecules. The last circumstance, at the least angles of  $\theta$ , attainable with existing experimental technique (25--30°), does not

permit the obtaining of a true initial slope of the curve  $(cH/I_0)_{c=0}$ .  
 In connection with this, as Sadron stressed as true in his review [2],  
 the data obtained by the method of light scattering for the molecular  
 weights and especially the dimensions of DNA molecules cannot be  
 considered sufficiently accurate. Sadron [2; 3] pointed out that the  
 asymptotic branch of the plot of dependency  $(C/I_0)_{c=0}$  on  $\sin \theta/2$   
 for solutions of DNA is linearly, which theoretically corresponds to  
 the case of rod-shaped particles (in contrast to Gaussian coils). At  
 the same time, the asymptote intercepts a negative segment on the axis  
 of ordinates, while for rod-shaped particles it is positive. In  
 Sadron's opinion, resting on the calculations of Luzzati and Benoit  
 [4], such a nature of the dependency  $(C/I_0)_{c=0}$  on  $\sin \theta/2$   
 testifies to the zigzag structure of the molecules of native DNA.  
 Luzzati and Benoit showed that for the asymptotes of the scattering  
 curve of solutions of particles, consisting of N linear freely-linked  
 segments with the length  $l$  each, the following relationship is correct

$$\begin{aligned}
 \left(\frac{cH}{I_0}\right)_{c=0} = & A \sin \frac{\theta}{2} + B \frac{1}{\lambda} \frac{NL}{M} \sin \frac{\theta}{2} + \\
 & + \frac{N}{\pi^2 M} \left[ 2 - \frac{\pi^2}{2} \frac{N-1}{N} \right],
 \end{aligned} \tag{1}$$

where H -- the optical constant of the solutions, determined by the  
 length of the light wave  $\lambda$  and the indices of refraction of the

particles and the solvent;  $M$  -- the molecular weight of the particles under study (macromolecules). The difference from a Gaussian freely-linked chain consists of the fact that in the given case  $l$  cannot be considered much less than  $\lambda$ . When  $N = 1$  the relationship (1) turns into the known formula for the asymptote of the scattering curve of rod-shaped particles  $\overline{[I]}$ :

$$\left(\frac{cH}{I_0}\right)_{c=0} = \frac{4}{\lambda} \frac{L}{M} \sin \frac{\theta}{2} + \frac{2}{\pi^2 M} \quad (2)$$

It is easy to see that with  $N \gg 2$  the second member  $B$  of the relationship (1) is negative.

Recently Ptitsyn and Fedorov  $\overline{[5]}$  established that the zigzag shaped model of the macromolecule is not unique, guaranteeing a negative initial ordinate of the asymptote with the curve  $(c/I_0)_{c=0}$ . As was demonstrated by these authors, for solutions of vermiform molecules, the stiffness of which is characterized by the value of the persistence line  $a \overline{[6]}$ , the second member  $B$  of the relationship (1) for the asymptote with the scattering curve  $(cH/I_0)_{c=0}$  takes the form

$$B = \frac{2}{\pi^2 M} - \frac{2}{3\pi^2 \mu a} \quad (3)$$

where  $\mu = M/L$  -- the mass (molecular) per unit of length of the particle, the complete length of which is  $L$ . For molecules of DNA,  $L \gg a$ , from where  $B < 0$ . The first member  $A$  for solutions of vermiform chains equals  $(\frac{4}{\lambda \mu} \sin \frac{\theta}{2})$  and conforms with the appropriate

member in the relationship (1), if it is taken into consideration that  $N l = L$  and  $N l / M = \mu^{-1}$ . In this manner Ptitsyn and Fedorov showed that a negative ordinate of the asymptote of the plot  $(eH/I_0)_{c=0}$  on  $\sin \theta/2$  does not solve the problem of the preferableness of the zigzag form or vermiform model of the DNA molecule. For either model, based on the slope of the asymptote it is possible to determine the value  $\mu = M/L$ . Since for a molecule of native DNA the first term in (3) is considerably less than the second, then based on the initial ordinate of the asymptote it is possible to also calculate the repeat length of the chain,  $a$ .

For solutions of Gaussian coils the asymptotic expression of the inverse value of the scattering function  $P^{-1}(\theta)$  is proportional, as is known  $[1]$ , to  $\sin^2 \theta/2$  and not  $\sin \theta/2$ . However, in the case of coils of sufficiently large dimensions it is very difficult to establish in an experiment the true nature of the dependency of the function  $P^{-1}(\theta)$  on the angle  $\theta$  in its asymptotic segment. This is illustrated in figure 1 on which the dependency  $P^{-1}(\theta)$  on  $\sin^2 \theta/2$  (curve 1) is plotted for very large Gaussian coils  $((\bar{R}^2)^{1/2} = 2000 \text{ \AA})$ . Beginning with the angle  $\theta = 30^\circ$ , curve 1 already yields an asymptotic branch where  $P^{-1}(\theta) \sim \sin^2 \theta/2$ . Curve 2 of figure 1 depicts the same value of  $P^{-1}(\theta)$  as the function of  $\sin \theta/2$ . Points, corresponding to the angles  $\theta \geq 90^\circ$ , lie very close to the straight line 3, having a negative initial ordinate. Consequently, in plotting the value  $(eH/I_0)_{c=0}$  for solutions of such large Gaussian coils, as the function  $\sin \theta/2$  it is possible (even with not too great a scattering of experimental points) to obtain a straight line with a negative initial ordinate for points  $\theta \geq 90^\circ$ .

It is especially simple to obtain the asymptotic branch of the curve  $(cH/I_\theta)_{c=0} \sim \sin \theta/2$  for solutions of large polydisperse coils, since polydispersity lessens the slope of the asymptotic segment of plot  $P^{-1} (\sin^2 \theta/2)$  and may even lead to its twisting [1; 7].

In this manner, the negative initial ordinate of the asymptotic straight line of the plot of dependency of the value  $(cH/I_\theta)_{c=0}$  on  $\sin \theta/2$  may be obtained both for solutions of zigzag formed and vermiform macromolecules, as well as for sufficiently large Gaussian coils (particularly polydisperse, and the samples of DNA are evidently polydisperse [2]).

More specific designations may yield the slope of the asymptotic straight line: It is possible to compare the value  $\mu = M/L$  obtained from the slope with data obtained by other methods.

We measured the light-scattering of native DNA solutions obtained from the thymus of a calf according to the method of Mirsky and Pollister by the authors of the work [8]. The measurements were performed on a photoelectric nephelometer "Sofika" in an interval of angles  $\theta$  of 30--150° and in an interval of DNA concentration of 0.02 -- 0.003%. The length of the light wave was 5460 Å. The initial concentration of DNA in the preparation was controlled by the spectrophotometric method proposed by Spirin [9]. The nativeness of the initial DNA was confirmed by measurements of ultraviolet (UV) absorption. The measurements were made in an aqueous 0.2 n solution of NaCl. Two independent series of light-scattering measurements were made with the sample of native DNA. The molecular weight  $M_w$  was determined from the plots of double extrapolation; in the two tests

they were equal to  $16.6 \cdot 10^6$  and  $14.3 \cdot 10^6$ . One of the graphs is presented in figure 2. Based on the slope of the asymptotic straight line of the plots of dependency  $(cH/I_0)_{c=0}$  on  $\sin \theta/2$  (figure 3), we determined the value of  $\mu = M/L$ , which proved to be equal correspondingly to  $240 \text{ 1/\AA}$  and  $290 \text{ 1/\AA}$ . Such a value of  $\mu$  agrees with  $\mu \approx 200 \text{ 1/\AA}$ , determined by the known model of Crick-Watson. In the experimental data cited by Sadron [2], the near value  $\mu \approx 220\text{--}250 \text{ 1/\AA}$  is figured. Based on the initial ordinate of the asymptote B we calculated, according to the relationship (3), the repeat length  $a$ , which in the two tests equaled 180 and 200  $\text{\AA}$ . The experimental data of other authors, processed in the article [5], yield the values  $a \approx 220\text{--}320 \text{ 1/\AA}$ . In our calculations of the value of  $a$ , we used the value  $\mu$ , obtained in the same experiment based on the slope of the asymptote. The use of  $\mu = 200 \text{ 1/\AA}$  (as in the work [5]) leads to a certain increase of the value of  $a$ , since from (3) it follows (for large M's) that  $a \sim 1/\mu$ . The value  $a \approx 200\text{--}300 \text{ 1/\AA}$ , as is noted in the work [5], agrees with the appraisal ( $\approx 360 \text{ 1/\AA}$ ) made on the basis of sedimentation data.

We also measured the light-scattering of solutions of one of the products from the ultrasonic destruction of the initial native DNA, obtained in the work [8]. We determined the molecular weight by the graph of double extrapolation; it turned out to be equal to  $1.6 \cdot 10^6$ . The plot of dependency of  $(cH/I_0)_{c=0}$  on  $\sin \theta/2$  is presented by curve 3, figure 3. Based on the slope of the asymptote  $\mu = 300 \text{ 1/\AA}$  was calculated, which agrees with the value of  $\mu$ , obtained by us for native DNA (the fragments of native DNA molecules were not subjected to despiralization in the process of destruction) (see [8]).



When calculating the persistence length  $a$  from the relationship (3), in this case one cannot omit the term  $2/\pi^2 M$ , since  $M$  is not sufficiently great. Substitution in (3) of  $M = 1.6 \cdot 10^6$  and the appropriate values of  $\mu$  and  $B$  lead to  $a = 1000 \text{ \AA}$ . Apparently such a result is connected with the polydispersity of the destroyed sample. In the presence of polydispersity a number average molecular weight  $M_n$  [5] should stand in the first term of the relationship (3). The molecular weight, in the event of considerable polydispersity, may be different by several times from the weight average  $M_w$  used by us, which approximately as much again will increase the calculated value of  $a$ .

The values, obtained from the plots of double extrapolation, of the root mean square radii of gyration  $(\bar{R}^2)^{1/2}$  of our samples of native and destroyed DNA (table) compare appropriately with the  $(\bar{R}^2)^{1/2}$ , calculated by the relationship

$$(\bar{R}^2)_Z^{1/2} = 2.7 \cdot 10^{-9} N^{0.58}, \quad (4)$$

established in [10] on the basis of experimental data obtained in the work [11]. We obtained the  $(\bar{R}^2)_Z^{1/2}$  for native and destroyed DNA correspondingly of 3500 (average for two tests) and 1200  $\text{\AA}$ , while the relationship (4) gives 3900 and 1080  $\text{\AA}$ . The conformity is fully satisfactory if it is taken into consideration that the method of double extrapolation cannot lay claim to great accuracy in the application for solutions of such extended molecules.

An interpretation of the light-scattering of solutions of degraded DNA on the basis of a model of a zigzag formed chain makes it possible to calculate from the relationship (1) the value  $N$  based on

a known B and M. Such a calculation leads to  $N = 2.2$ . This means that the molecule consists of two-three straightline segments. With a molecular weight  $= 1.6 \cdot 10^6$  such a value of N agrees with the molecular weight  $M \approx 6 \cdot 10^5$ , at which, according to the data of Sadron [3], the light scattering of DNA fragments acquires an asymptotic behavior, characteristic for rod-shaped particles (positive initial ordinate), that is, with the molecular weight of one segment of the zigzag.

Denaturation is carried out by heating a solution with a concentration, the concentration for measurement of light-scattering, for 30 minutes at  $100^\circ$ . After thorough heating the solution is rapidly cooled to room temperature. The graph of double extrapolation for denatured DNA is shown in figure 4.

The molecular weight  $M_w = 8.3 \cdot 10^6$  of denatured DNA within the limits of measurement errors turns out to be half the molecular weight of native DNA --  $15.5 \cdot 10^6$  (average from two measurements). This corresponds to the separation of the strands of the double spiral of DNA during its denaturation. The root mean square radius of gyration of coils of denatured DNA  $(\bar{R}^2)^{1/2} = 1860 \text{ \AA}$ . When utilizing this value for evaluating the flexibility of a chain of denatured DNA, polydispersity should be taken into consideration. Based on the "initial"  $S_0$  and the asymptotic  $S_\infty$  slope of the plot of dependency of  $(\epsilon H/I_\theta)_{c=c}$  on  $\sin^2 \theta/2$  (figure 4), it is possible to find, in agreement with the formula [7]:

$$\frac{S_0}{S_\infty} = \frac{2}{3} \frac{M_z}{M_w}, \quad (5)$$

the proportion  $M_z/M_w = 2.7$ . Keeping in mind that for the coils

$(\bar{R}^2)^{1/2} \sim M^{1/2}$ , we find

$$(\bar{R}^2)_w^{1/2} = (\bar{R}^2)_z^{1/2} \left( \frac{M_w}{M_z} \right)^{1/2} = 1130 \text{ \AA}.$$

As is known [12], the value of the statistical segment  $A$  of the coil-shaped chain molecule may be calculated, knowing the root mean square distance between its ends  $(\bar{h}^2)^{1/2}$  and the full length of the chain  $L$ :

$$A = \bar{h}^2 / L. \quad (6)$$

Since  $\bar{h}^2 = 6\bar{R}^2$ ,  $L = \frac{M}{M_0} b$  ( $M_0 = 330$  -- weight of a monomer link of the chain,  $b = 7.2 \text{ \AA}$  -- its dimensions along the chain), based on the measured values  $(\bar{R}^2)_w^{1/2}$  and  $M_w$  it is possible to calculate the dimension of segment  $A$ . In our case of denatured DNA, segment  $A$  turned out to be equal to  $43^\circ$  and contains six monomer units (mononucleotides). We recall that a segment of a chain of the most flexible synthetic and natural (rubber) polymers also contains six--eight monomer units [13]. Therefore, a chain of denatured DNA in solution possesses a flexibility corresponding to the flexibility of a molecule of rubber. If the flexibility of the chain is characterized by the value of the ratio  $\bar{R}_z^2 / M_w$ , then the result obtained by us,  $(\bar{R}_z^2 / M_w)^{1/2} = 0.65 \cdot 10^{-8}$ , compared well with the similar value  $0.61 \cdot 10^{-8}$ , obtained for the denatured DNA of calf thymus in the work [14].

## Conclusions

1. In an interpretation of the data on the light-scattering of DNA solutions, it must be kept in mind that the negative initial ordinate of the asymptotic straight line of the plot of dependency  $(\frac{cH}{I_0})_{c=0}$  on  $\sin \theta/2$  may satisfy not only the case of zigzag formed and vermiform macromolecules, but also Gaussian coils of sufficiently large dimensions.
2. A graphic interpretation of our measurements for native DNA and DNA which has been degraded by ultrasound in the coordinates  $(\frac{cH}{I_0})_{c=0}$  and  $\sin \theta/2$  lead to the reasonable values of  $\mu$ ,  $a$  and  $N$  during the utilization of both the vermiform and zigzag formed model of the molecule.
3. The measurements show that the flexibility of a chain of denatured DNA corresponds to the flexibility of rubber-like synthetic polymers.

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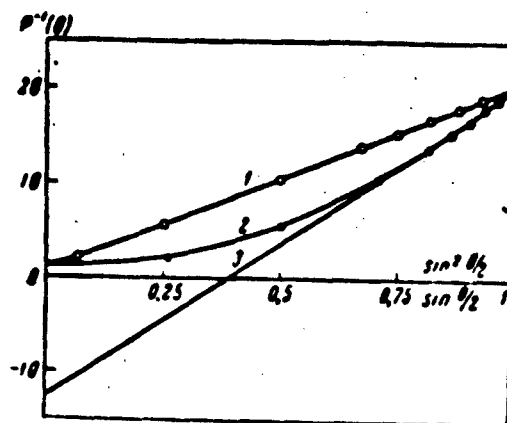


Figure 1. Graph of the reverse value of the function of scattering for large Gaussian coils ( $(\bar{R}^2)^{1/2} = 2000 \text{ \AA}$ ) of  $P^{-1}(\theta)$ , as the function  $\sin^2 \theta/2$  (curve 1) and  $\sin \theta/2$  (curve 2). Straight line 3 -- the asymptote of curve 2.

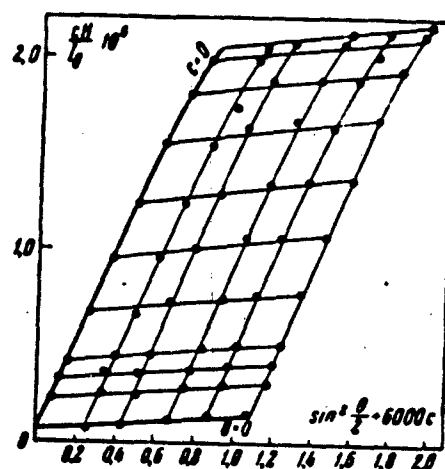


Figure 2. Plot of double extrapolation for native DNA of calf thymus ( $M_w = 15.5 \cdot 10^6$ )

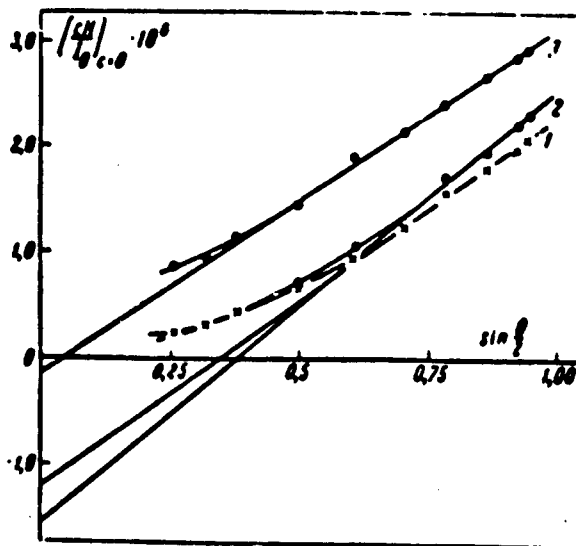


Figure 3. Plot of dependency of the value  $\left(\frac{cH}{T_0}\right)_{c=0}$  on  $\sin \theta/2$  for native (1 and 2) and destroyed (3) DNA of calf thymus.

#### Results of Measurements

Preparation of DNA	$M_w \cdot 10^{-6}$	$R_z, \text{\AA}$	$\mu, \text{\AA}^{-1}$	$a, \text{\AA}$
Native DNA				
1st series of measurements	16.6	3700	240	180
2nd series	14.3	3300	290	200
Denatured DNA	8.3	1860	—	—
Destroyed DNA	1.6	1200	300	1000

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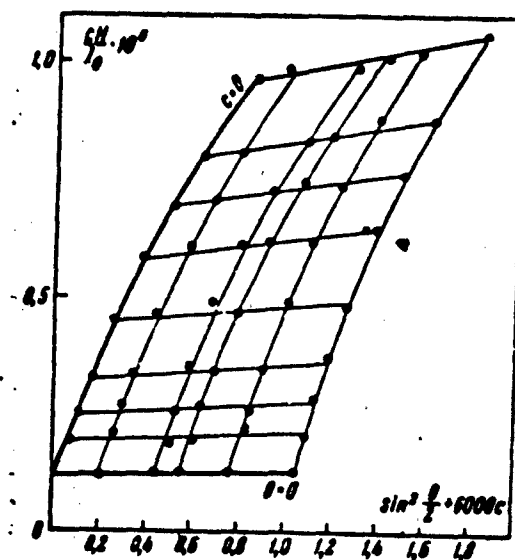


Figure 4. Plot of double extrapolation for denatured DNA of calf thymus ( $M_w = 8.3 \cdot 10^6$ )